Transformation of *Escherichia coli* by Plasmid DNA from *Erwinia stewartii*

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ABSTRACT

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Seven cryptic plasmids from *Erwinia stewartii* strain DC336 were transformed into *Escherichia coli* strain HB101 using indirect selection for R-plasmid pSC201. Restriction endonuclease digestions of these plasmids revealed that they are unique and the larger plasmids are not cointegrates or multimers of the smaller plasmids. Hybridization experiments showed that pDC150 and pDC170 sequences are conserved in similar-sized plasmids in other strains. pDC150 and pDC170 also contained a sequence(s) that hybridized weakly to many other *E. stewartii* plasmids.

Erwinia stewartii is an enteric bacterium that incites a disease in susceptible corn plants known as Stewart's wilt. *E. stewartii* is unusual because at least 20% of its genome is plasmid DNA; every virulent *E. stewartii* strain examined to date contains from six to 13 plasmids ranging in size from 4.1 to 318 kb (kilobase) (4). Most wild type strains contain 4.1, 13, 25, 45, 65, 74, and 103 kb plasmids. Plasmids in this species are cryptic, except for pDC250 (52 kb, from strain SW2) and pDC190 (103 kb, from strain SS104), which are conjugative (3,7). Because possessing so many plasmids is not characteristic of other *Erwinia* species, we wondered if all the plasmids in a strain were unique or if the large plasmids were cointegrates of the smaller ones. We also wanted to know if similar-sized plasmids from different strains were closely related. To answer these questions, it was first necessary to purify genetically the individual plasmids. This paper describes the isolation of seven *E. stewartii* plasmids by transformation into *Escherichia coli*, and their comparison by restriction endonuclease digestions and Southern blot hybridization.

MATERIALS AND METHODS

For this study, *E. stewartii* strain DC336, an Nal^r Mu *cts*62 lysogen of *E. stewartii* strain SS104 (4) that is lacking pDC160 (63 kb), was used as a transformation donor. DC336 contains the following plasmids: pDC100 (4.1 kb), pDC110 (4.1 kb), pDC120 (13 kb), pDC130 (25 kb), pDC140 (34 kb), pDC150 (45 kb), pDC170 (65 kb), pDC180 (74 kb), pDC190 (103 kb), and pDC200 (318 kb). Other wild-type *E. stewartii* strains (shown in Fig. 4) have been previously described (4,7). pSC201 (8) was obtained from S. N. Cohen, Stanford University.

Large-scale isolation of plasmid DNA was done using the alkaline denaturation method of Currier and Nester (6) as modified by Currier and Morgan (5). Total plasmid DNA from strain DC336 was cotransformed into competent cells (9) of *E. coli* strain HB101 using indirect selection for the tetracycline resistance marker on pSC201 (8).

Procedures for rapid plasmid isolation, agarose and acrylamide gel electrophoresis, nick-translation of DNA with [^{32}-P]-dCTP,

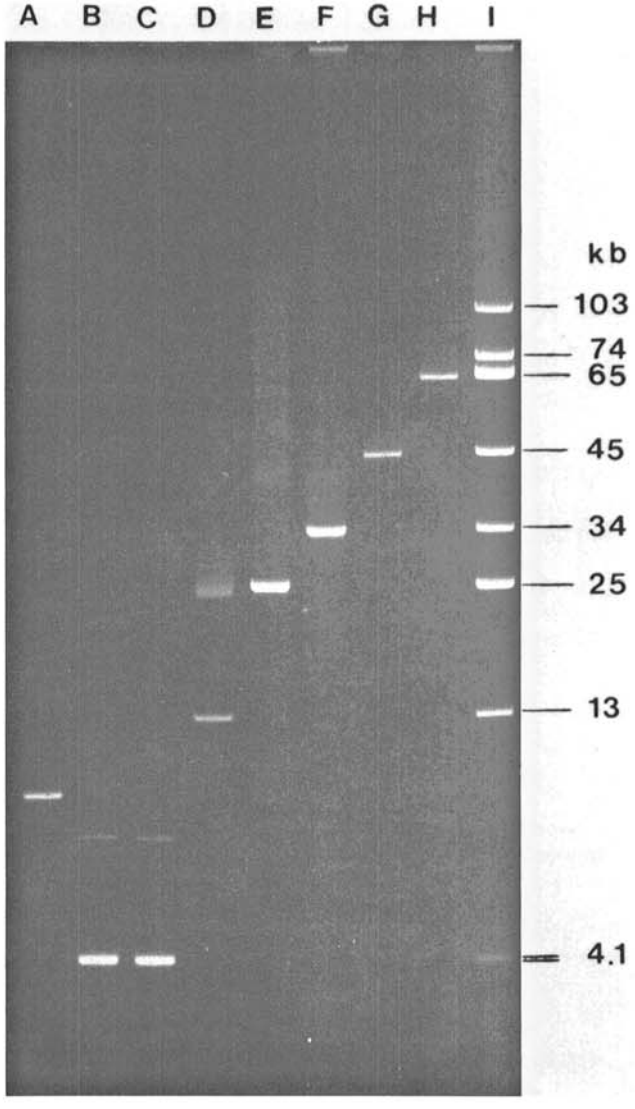

Fig. 1. Agarose gel (0.5% w/v) of *Erwinia stewartii* strain DC336 plasmids transformed into *Escherichia coli*: A, pSC201; B, pDC100; C, pDC110; D, pDC120; E, pDC130; F, pDC140; G, pDC150; H, pDC170; and I, DC336 total plasmid DNA.

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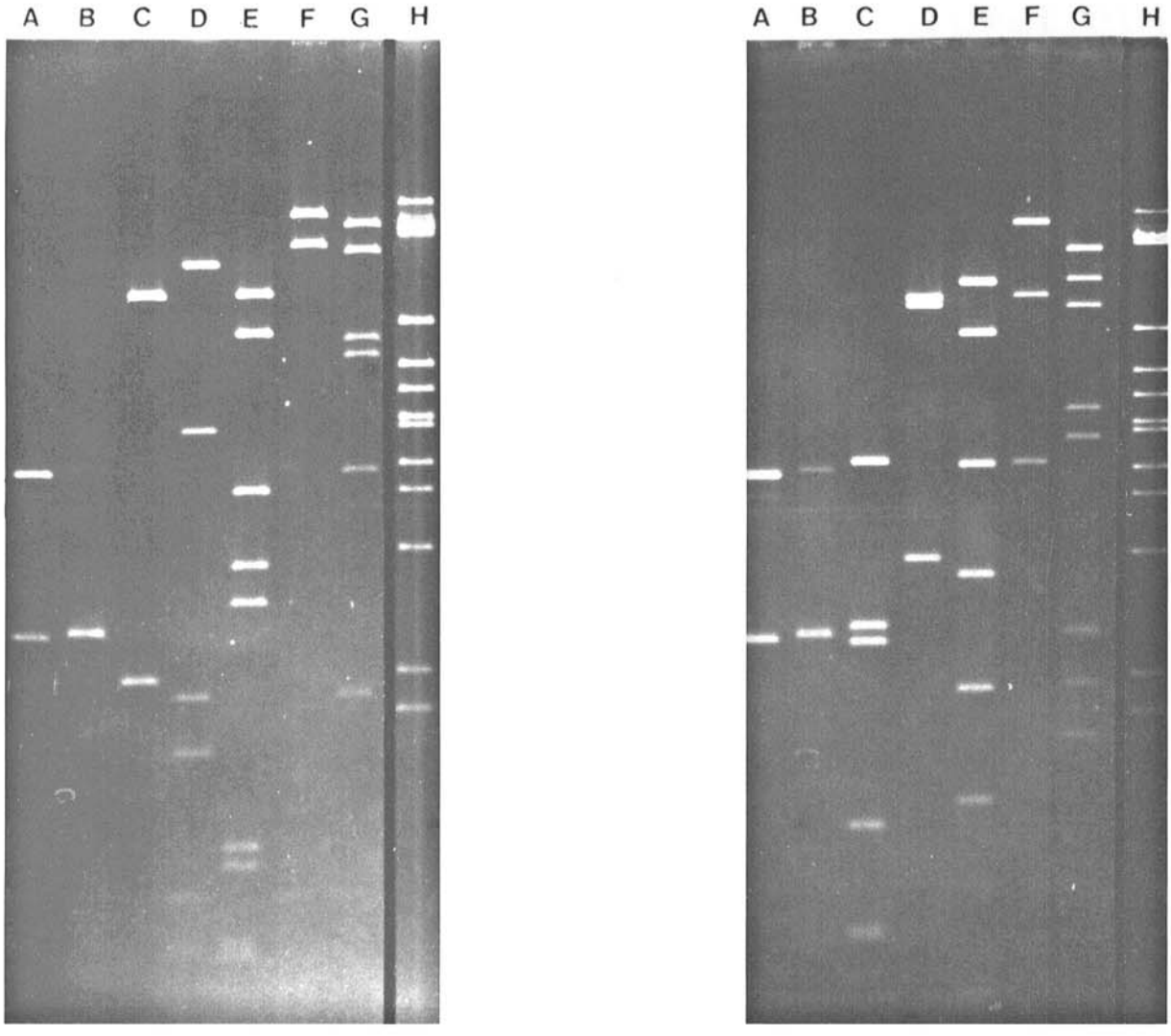

Fig. 2. Agarose gels (0.8% w/v) of *Erwinia stewartii* strain DC336 plasmids transformed into *Escherichia coli* restricted with *Eco*R1 (left) and *Hin*dIII (right): A, pDC100; B, pDC110; C, pDC120; D, pDC130; E, pDC140; F, pDC150; G, pDC170; and H, a mixture of uncut λ DNA, *Eco*R1 digested λ DNA, and *Hin*dIII digested λ DNA. pDC100 and pDC110 were not cut by either enzyme and the bands shown are CCC and OC forms of the plasmid.

Southern blot hybridizations and autoradiography have been previously described (1,2,7–9). Restriction enclonucleases and a nick-translation kit were obtained from BRL and used according to their recommendations.

RESULTS AND DISCUSSION

E. coli HB101 (9) was transformed with a mixture of DC336 total plasmid DNA from DC336 and pSC201 DNA in a ratio of 500:1. Transformants were selected for tetracyline resistance and screened by agarose gel electrophoresis to detect any that contained plasmids from DC336 in addition to pSC201. Of 282 tetracycline-resistant colonies screened, 17 received plasmids from DC336. Transformants containing pDC100, pDC110, pDC120, pDC130, pDC140, pDC150, and pDC170 were obtained. pSC201, which is temperature sensitive for replication (*ts*), was eliminated from the transformants by growth at 45 C, thereby leaving only the *E. stewartii* plasmid in the strain. *E. coli* HB101 strains transformed with pDC100, pDC110, pDC120, pDC130, pDC140, pDC150, and pDC170 that have been cured of pSC201 are shown in Figure 1.

To determine if individual plasmids in DC336 were unique, the isolated plasmids were digested with *Eco*RI, *Bam*HI, *Hin*dIII, and *Kpn*I. The *Eco*RI and *Hin*dIII digests are shown in Figure 2. pDC100 and pDC110 did not contain sites for *Eco*RI and *Hin*dIII but could be distinguished by *Hae*III (Fig. 3) and *Bam*H1, which does not cut pDC110 and cuts pDC100 twice. An overall comparison of the various plasmid restriction digests indicates that each of the plasmids had different restriction profiles with no apparent fragments in common. Thus, the larger plasmids in DC336 did not appear to be cointegrates or multimers of the smaller plasmids in this strain.

The transformed *E. coli* strains allowed us to answer the questions of whether similar-sized plasmids in different strains were, in fact, homologous, and if the unique plasmids found in some strains were related to any of the conserved plasmids found in *E. stewartii*. We previously reported (2) that pDC250 hybridizes to all other 52 kb plasmids in *E. stewartii* strains harboring that size plasmid (strains SW2, SW3, SW11, and and SW14) and to pDC140 (34 kb) in strain DC336. The degree of homology is quite extensive between pDC250 and pDC140 as determined by multiple restriction digests and Southern blot hybridizations. Similarly, we demonstrated that pDC191 (a derivative of pDC190) hybridizes to all other 103 kb plasmids and four apparent deletion plasmids of 79, 71, 68, and 62 kb (7). In this study, we extended these observations to pDC150 and pDC170. pDC150 probe DNA not only hybridized strongly with other 45 kb plasmids in all six *E. stewartii* strains tested (SW2, SW3, SW14, SW20, DC211, and SW11; Fig. 4), but it also hybridized weakly with almost all the other plasmids in these strains. This implies that pDC150 contains a sequence(s), such as an IS element, that is also present in many

other *E. stewartii* plasmids. [^{32}P]-labelled pDC170 hybridized with the 65 kb plasmids in the five *E. stewartii* strains tested (SW2, SW3, SW14, SW20, and DC211) that contained this size plasmid (Fig. 4). Strain SW11, which did not contain a 65 kb plasmid, instead had two plasmids of approximately 42 and 29 kb that hybridized strongly with the pDC170 probe (Fig. 4). When pDC170 probe DNA was hybridized to a blot of SW11 total plasmid DNA restricted with *Bam*H1, *Eco*R1, *Hin*dIII, or *Eco*R1 plus *Hin*dIII (Fig. 5), most homologous fragments corresponded in size to those of pDC170. These results indicate conservation of pDC170 sequences in SW11 and suggest that the 41 and 29 kb plasmids in this strain share a common origin with pDC170.

Although multiple plasmid carriage is known in the Enterobacteriaceae, it is usually characteristic of individual strains rather than a species. Why *E. stewartii* has evolved such a complex plasmid system is a mystery. The results of this study confirm our notions that each plasmid in a strain is different from the others

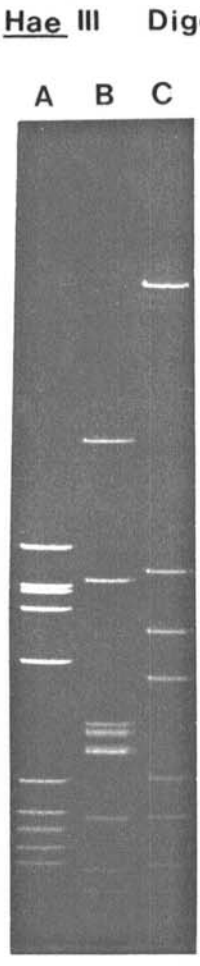

Fig. 3. Acrylamide gel (4.0% stacking gel, and 7.5% separating gel) of a *Hae*III restriction digest of: A, pBR322; B, pDC100; and C, pDC110.

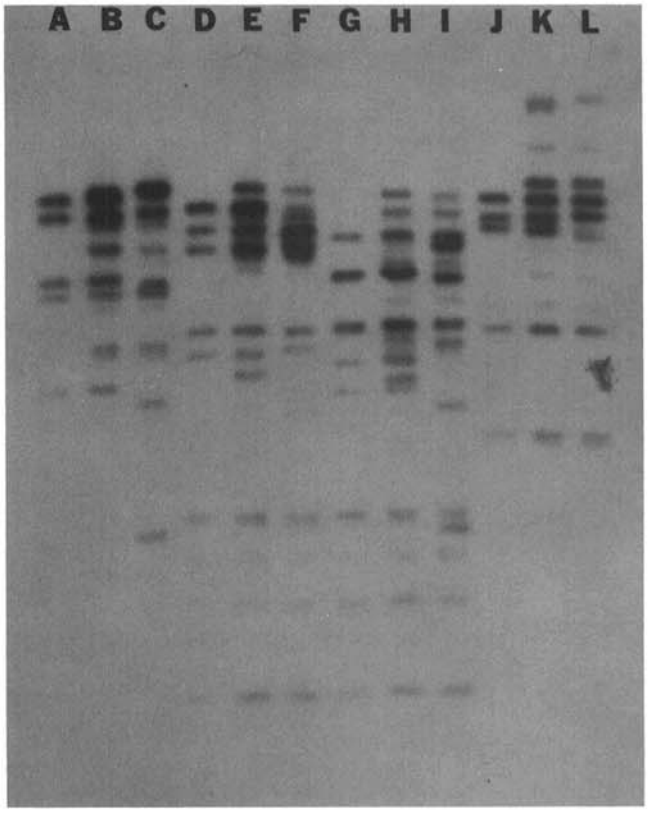

Fig. 5. Southern blot hybridization of plasmid DNA from *Erwinia stewartii* strains SS104 and SW11 with [^{32}P]-labeled pDC170 probe. A, D, G, and J, pDC170; B, E, H, and K, SS104; C, F, I, and L, SW11. Plasmid DNA was digested with *Eco*R1 (A-C), *Hind*III (D-F), *Eco*R1 plus *Hind*III (G-I), and *Bam*H1 (J-L).

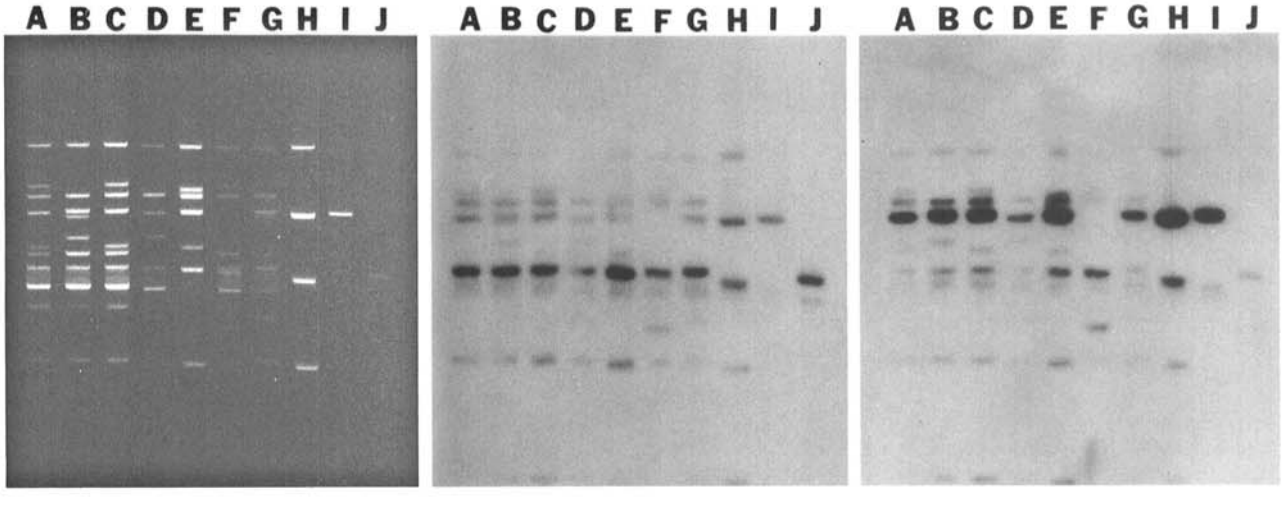

Fig. 4. Hybridization of plasmids from *Erwinia stewartii* strains with [^{32}P]-labeled pDC150 and pDC170 probes. Agarose gel (left) and Southern blots probed with pDC150 (middle) and pDC170 (right) of: A, SW2; B, SW3; C, SW14; D, SW20; E, DC211; F, SW11; G, SS104; H, DC350; I, pDC170; and J, pDC150.

and the plasmids are highly conserved between strains. The transfer of most of these plasmids to a defined genetic background reported here should facilitate screening for phenotypic traits. Perhaps this will reveal what advantage there might be in maintaining so many genes as plasmids rather than chromosomal DNA. Certainly the plasmid system of *E. stewartii* represents a coadapted group of genes, which may include normally chromosomal essential genes to stabilize the system. A need for such plasmid stability could be the periodic alternation of ecological niches that *E. stewartii* undergoes. This bacterium must be able to colonize the alimentary tract of the corn flea beetle (*Chaetocnema pulicularia* Melsh.) in order to overwinter and it must be able to infect corn plants between generations of beetles. Thus traits that enable it to grow in plants and insects must be stable for many generations in the absence of phenotypic selection.

LITERATURE CITED

1. Birnboim, H. C., and Doly, J. 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. 7:1513-1523.
2. Coplin, D. L., Frederick, R. D., and McCammon, S. L. 1985. Characterization of a conjugative plasmid from *Erwinia stewartii*. J. Gen. Microbiol. 131:2985-2991.
3. Coplin, D. L., and Rowan, R. G. 1978. Conjugative plasmids in *Erwinia stewartii*. Pages 67-73 in: Proceedings of the 4th International Conference on Plant Pathogenic Bacteria. Angers, France, 1978. Station de Pathologie Végétale et Phytobactériologie, Institute National de Recherche Agronomique, Angers, France.
4. Coplin, D. L., Rowan, R. G., Chisholm, D. A., and Whitmoyer, R. E. 1981. Characterization of plasmids in *Erwinia stewartii*. Appl. Environ. Microbiol. 42:599-604.
5. Currier, T. C., and Morgan, M. K. 1981. Restriction endonuclease analysis of the incompatibility group P-1 plasmids RK2, RP1, RP4, R68, and R68.45. Curr. Microbiol. 5:323-327.
6. Currier, T. C., and Nester, E. W. 1976. Isolation of covalently closed circular DNA of high molecular weight from bacteria. Anal. Biochem. 76:431-441.
7. Frederick, R. D., and Coplin, D. L. 1986. Plasmid pDC190: Distribution among *E. stewartii* strains and nonassociation with virulence. Phytopathology 76:165-170.
8. Kretschmer, P. J., Chang, A. C. Y., and Cohen, S. N. 1975. Indirect selection of bacterial plasmids lacking identifiable phenotypic properties. J. Bacteriol. 124:225-231.
9. Maniatis, T., Fritsch, E. F., and Sambrook, J. 1982. Molecular cloning: A laboratory manual. Cold Spring Harbor Laboratory. Cold Spring Harbor, NY. 545 pp.